Relation of growth hormone response to growth hormone-releasing hormone to estimation of milk production via deuterium oxide dilution in beef cattle

T. L. Auchtung, D. J. Baer, R. A. Erdman, S. M. Barao and G. E. Dahl

J Anim Sci 2002. 80:1270-1274.

Relation of growth hormone response to growth hormone-releasing hormone to estimation of milk production via deuterium oxide dilution in beef cattle[1]

T. L. Auchtung*, D. J. Baer†, R. A. Erdman*, S. M. Barao*, and G. E. Dahl*[2]

*Department of Animal and Avian Sciences, University of Maryland, College Park 20742-2311 and
†USDA, ARS, Beltsville Human Nutrition Research Center, Beltsville, MD

ABSTRACT: Current methods of estimating milk production in beef cows can be time-consuming, labor-intensive, and subject to high variability. The weigh-suckle-weigh (WSW) method requires repeated separation of offspring from their dams. Machine milking requires that animals be acclimated to the equipment prior to the estimation. The objective of Exp. 1 was to validate a deuterium oxide (D_2O) dilution method of estimating milk production in cattle. In Exp. 1, Holstein calves (n = 5) averaging 29 ± 2 d of age and 52.6 ± 2.5 kg (± SE) were used as the model. Blood was collected for baseline D_2O measurements followed by an injection of 300 mg D_2O/kg BW. Syringes were weighed before and after the injection to gravimetrically determine the dose. Another blood sample was collected after D_2O was allowed to equilibrate with body water for 2 h, and on each of the next five consecutive days, prior to feeding. Actual milk intake was measured by disappearance (i.e., amount of milk replacer offered to the calf minus the amount refused). Deuterium oxide in plasma was measured by mass spectrometry and milk intake was computed from the disappearance curve of D_2O in blood plasma for each calf. Accumulated milk intake estimated by D_2O dilution was highly correlated ($y = 0.9x + 0.6$; $R^2 = 0.99$; $P < 0.001$) with actual milk intake. The objectives of Exp. 2 were to determine whether 1) D_2O dilution was comparable to a standard measure of milk production in beef heifers and 2) growth hormone (GH) response to GH-releasing hormone (GHRH) in heifers at weaning is predictive of subsequent milk production. Deuterium oxide dilution and WSW were compared using 14 first-calf Angus heifers and their calves. Deuterium oxide dilution was used to estimate milk production of 40 first-calf Angus heifers that had been challenged with GHRH at weaning. Results indicate that the D_2O dilution method is correlated ($R^2 = 0.89$; $P = 0.04$) to the WSW estimation of milk production. Growth hormone response to GHRH in weanling heifers is positively related ($R^2 = 0.22$; $P = 0.03$) to their subsequent milk production. Deuterium oxide dilution in calves offers an additional approach to the estimation of milk production of the dam in typical beef cattle production settings.

Key Words: Deuterium Oxide, Milk Production, Somatoliberin

 J. Anim. Sci. 2002. 80:1270–1274

Introduction

Milk production is a primary factor influencing the weaning weight of beef calves (Beal et al., 1990). Because of the importance of the somatotropic axis in ruminant milk production, GH is a candidate as a physiological predictor of lactational potential. Previous observations (Auchtung et al., 2001a) indicate that milk yield in mature beef cows is related to GH response to GHRH. However, whether such a relationship can be predicted prior to calving is unknown.

Lack of a reliable and low-cost method of measuring milk production in individuals limits the development of a method of predicting milk yield in beef cattle. Traditional methods for estimating milk consumption by calves include the weigh-suckle-weigh (WSW) method and machine milking. Both are widely accepted and yield fair estimates of milk yield, yet each has disadvantages. Isotope dilution is used to estimate milk consumption and production in many species (Pettitgrew et al., 1985; Oftedal et al., 1983), including ruminants (Dove and Freer, 1979; McEwan and Whitehead, 1971).

[1]Supported by the Maryland Agric. Exp. Sta. grant AASC-99-42 awarded to G. E. Dahl. Bovine GHRH (1-30) analog was generously provided by W. M. Moseley of Pharmacia & Upjohn. The authors thank Bob Staples for his help with the deuterium oxide sample analysis. Thanks to Erin Duffy and the farm crew at the Clarksville facility for their help with experiment 1 and Eddie Draper, Kevin Morgan, Frank Walbert, J. W. Dulin, and Lisa Yoash for their help with data collection for experiment 2 at the Wye Research and Education Center. Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

[2]Correspondence and current address: 230 Animal Sciences Laboratory, MC-630, University of Illinois, Urbana, IL 61801 (phone: 217-244-3152, fax: 217-333-7088, E-mail: gdahl@uiuc.edu).

Received August 14, 2001.
Accepted November 20, 2001.

In cattle, previous research in isotope dilution used tritiated water (Dove and Axelson, 1979). No studies in cattle, however, compare the dilution method to standard milk estimation techniques. Due to the general disadvantages of radioisotope use and the lack of validation against standard methods, an alternative approach is needed. An alternative method for estimating milk yield is through deuterium oxide (D_2O) dilution. As a stable isotope of water, D_2O does not present the waste-handling problems typical of radioisotopes.

The objective of Exp. 1 was to determine the validity of D_2O dilution for estimating milk intake by calves. Estimates obtained from D_2O dilution were compared to accurate, physical measurements of the intake of dairy calves. In Exp. 2, the objectives were to validate D_2O dilution in beef cattle by comparison to WSW estimations, and to determine whether GH response to GHRH in weanling heifers is related to their subsequent milk production.

Materials and Methods

Animals

Experiment 1. Five Holstein calves (two bulls and three heifers) housed at the Central Maryland Research and Education Center (Clarksville, MD) were used to evaluate the D_2O dilution method of measuring milk intake. Milk replacer (Land O'Lakes, Inc., Fort Dodge, IA) was fed twice daily. Calves were weighed on the day preceding the start of the experiment. Average age and weight at the start of the experiment were 29 ± 2 d and 52.6 ± 2.5 kg (± SE), respectively. The calves were housed individually in pens (1.8 × 3.0 m) under natural photoperiod. Individual milk, water, and grain intake was measured daily. Water and grain intake was negligible in comparison with milk intake. Milk intake was determined by the disappearance of the milk replacer and by the D_2O dilution method over a period of 5 d.

Experiment 2. Forty Angus calves housed at the Wye Research and Education Center (Queenstown, MD) were used for this experiment. Calves were selected for this experiment on the basis of their dam's GH response to GHRH (described below). Calves were raised solely by their dams on either orchardgrass/alfalfa or fescue/red clover pasture; no creep feed was offered. Water was accessible at all times. Calves were weighed immediately before collection of the baseline blood sample and D_2O injection. The D_2O dilution procedure was performed twice between birth and weaning. The first measurement was made at an average of 39 ± 1 d of age (± SE) and 52.9 ± 0.9 kg BW, an age when most young ruminants are functionally monogastric and dependent solely on milk for nutrients. The second measurement was performed at 67 ± 0.3 d of age and 76.0 ± 0.3 kg BW, which is approximately the time of peak lactation for Angus cows (Jenkins and Ferrell, 1992). It was assumed that the main intake of water was via milk, and, therefore, milk ingestion could be estimated by measuring total water turnover using D_2O dilution. Calves were grouped by age (11 to 15 animals/group) to reduce the total time of separation from their dams during the D_2O dilution procedure and the time between weighing and suckling during the WSW procedure.

Deuterium Oxide Dilution Procedure

Experiment 1. On d 1 of the experiment, conducted in late September 1999, two baseline samples of blood (10 mL) were collected into sterile Vacutainer heparinized plasma tubes (Becton Dickinson and Co., Franklin Lakes, NJ) from the jugular vein of calves restrained individually. Calves were then given an i.v. injection of 300 mg of D_2O/kg BW (99.9%, Sigma-Aldrich Co., St. Louis, MO). Deuterium oxide was salinized with 0.9% NaCl. Time and duration of injections were recorded. Syringes were weighed before and after the injection to the nearest 0.1 g to gravimetrically determine the actual dose. The D_2O was allowed to equilibrate with body water for 2 h (Macfarlane et al., 1969; Yates et al., 1971) before two blood samples were collected and calves were fed. Actual milk intake was calculated by disappearance, that is, the amount of milk offered to the calf minus the amount of milk refused by the calf. On each of the next 5 d, two blood samples were collected immediately before the morning feeding. The time at which each sample was collected was recorded to the nearest 30 s.

Experiment 2. The D_2O dilution procedure in this experiment was identical to the procedure for experiment 1, except that only one blood sample was collected each day from each calf, due to the larger number of animals. Following the 2 h equilibration and subsequent blood sample, calves were returned to their dams. On each of the five consecutive days of blood collection, calves were removed from their dams only during the blood collection period, which did not exceed 30 min.

GHRH Challenge (Exp. 2)

Sixty-seven Angus weanling heifers were challenged with GHRH in October 1998, as described by Auchtung et al. (2001b). Briefly, each heifer received two challenge doses (1.5 and 4.5 μg/100 kg BW) of a bovine GHRH (1-30) analog (Pharmacia & Upjohn, Kalamazoo, MI) in a randomized crossover design. Blood was collected immediately before and 10 min after the injection of GHRH. Growth hormone was analyzed in serum using a RIA validated by Connor et al. (2000). Of the 67 heifers challenged with GHRH, 40 heifers remained in the herd and successfully calved. Those heifers and their calves were used in this study to determine the relationship of GH response to GHRH with estimates of milk production.

Weigh-Suckle-Weigh Procedure (Exp. 2)

Of the 40 heifers mentioned previously, 14 heifers and their calves were selected for the weigh-suckle-

weigh procedure, which was performed in addition to the D_2O dilution procedure in experiment 2. Calves were separated from their dams at 1400, returned to their dams to nurse at 2000, and separated from their dams until 0800 the next day. This initial 6-h separation period was performed to empty the udder and minimize the variation among the dams. Because we could not be sure when each of the calves had last suckled, it was important to empty all of the dams' udders so that each animal was starting from a similar stage in her daily milk production, thereby minimizing variation in estimated milk production among the dams. After the 12-h separation period, calves were weighed to the nearest 0.5 kg, allowed to nurse for no more than 30 min, and weighed again. Calves were then returned to the main groups of calves for the D_2O dilution procedure. Prenursing weight of the calf was subtracted from postnursing weight to obtain an estimate of the 12-h milk production of the dam. The 12-h estimate was doubled to estimate milk production for 24 h, which was the value used for comparison with the D_2O dilution procedure estimate of milk production. All animal procedures were approved by the University of Maryland Animal Care and Use Committee.

Analysis of D_2O and Milk Yield Estimation (Exp. 1 and 2)

Plasma was analyzed for D_2O using continuous flow isotope ratio mass spectrometry (Europa Hydra ANCA GSL model, PDZ Europa Ltd., UK). Sample (0.5 mL) was added to a gas exetainer tube (PDZ Europa Ltd.), after which a glass vial containing platinum on alumina catalyst powder (Sigma-Aldrich Co.) was also placed in the tube. The exetainer tubes were then evacuated and filled with hydrogen. Samples and reference water tubes were allowed to equilibrate at room temperature for at least 3 d. Samples were then analyzed using the HYDRA-H_2O system of the mass spectrometer. Concentrations of D_2O, initially expressed relative to standard mean ocean water, were converted to parts per million. Estimated milk intake was computed from the disappearance curve of D_2O in plasma for each calf. The disappearance curve includes the concentration of D_2O in the baseline blood samples, which allows for D_2O naturally present in the animal's body. Deuterium oxide was then injected and measured in subsequent blood samples. Deuterium oxide leaves the body as water taken in and "turned over"; thus, the original concentration of D_2O is diluted. The rate of this turnover is important in estimating final milk production. The equation used to describe the disappearance curve was $Y = A + Be^{-kt}$, where Y is D_2O concentration (mg/L), A is the baseline concentration of D_2O, B is the intercept in mg/L, k is the rate constant, and t is the time in hours. The optimizer option of Quattro Pro (Corel Corp. Ltd., Dublin, Ireland) was used to obtain the parameter estimates of the equation for each calf. Liters of water turned over per hour was estimated using the equation

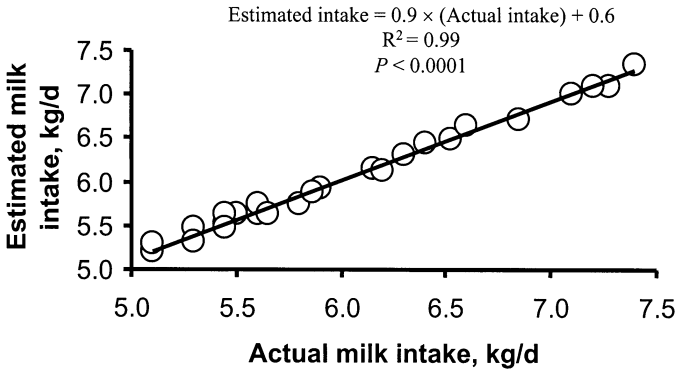

Figure 1. Relationship between actual milk intake of Holstein calves (n = 5) and milk intake estimated by the deuterium oxide (D_2O) dilution method over 5 d in Exp. 1.

Water = [(dose of D_2O × 1,000)/B] × k, where Water is the water turned over in liters per hour and dose of D_2O is in grams. The rate liters of water per hour was converted to kilograms of milk per hour using established conversion factors (Dove and Axelson, 1979; Prawirodigdo et al., 1990a).

Statistical Analyses

Statistical analyses were performed using the SAS System v. 6.12 (SAS Inst. Inc., Cary, NC). In Exp. 1, regression analysis was used to compare actual milk intake, as estimated by disappearance of milk replacer, to milk intake estimated by the D_2O dilution procedure. In Exp. 2, regression analysis was used to compare milk yield as estimated by the WSW procedure with milk yield estimated by the D_2O dilution procedure. Regression analysis was used to relate the GH response to GHRH with milk yield as estimated by the D_2O dilution procedure.

Results

Experiment 1

Milk intake as measured by disappearance averaged 5.86 ± 0.14 kg/d (± SE) and 5.88 ± 0.13 kg/d as estimated by the D_2O dilution procedure. Regression analysis of the milk consumption estimates yielded a regression equation of y = 0.9x + 0.6, where y = estimated intake and x = actual intake ($R^2 = 0.99$; $P < 0.0001$; Figure 1).

Experiment 2

Average milk production of the 40 first-calf heifers, as estimated by D_2O dilution, was 13.7 ± 0.53 kg/d. Of the 14 heifer/calf pairs on which WSW measurements were made, the average milk production was 10.7 ± 0.70 and 13.1 ± 0.86 kg/d as estimated by WSW and D_2O dilution, respectively. The equation of the regression analysis was y = 0.8x + 0.4, where y = milk yield esti-

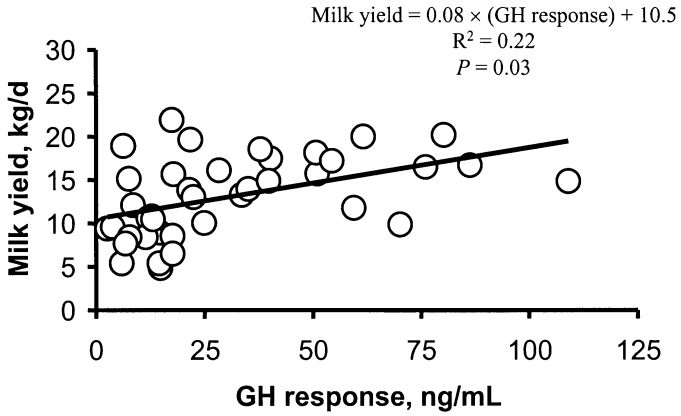

Figure 2. Relationship between GH response of Angus heifers (n = 40) at weaning to 1.5 (g GHRH/100kg BW vs milk yield as estimated by the deuterium oxide (D_2O) dilution method in Exp. 2.

mated by the D_2O dilution method and x = milk yield estimated by the WSW method ($R^2 = 0.89$; $P = 0.04$).

Heifers' GH response to the low dose of GHRH at weaning was positively related ($y = 0.08x + 10.5$; $R^2 = 0.22$; $P = 0.03$; Figure 2) to their milk production, as first-calf heifers, when estimated by the D_2O dilution procedure. The GH response to the high dose of GHRH (4.5 μg/100kg BW) was not related to any estimates of milk production.

Discussion

Growth hormone response to secretagogues, such as GHRH and thyrotropin-releasing hormone (**TRH**), is related to indices of lactational performance in cattle. For example, relative GH response to TRH is positively associated with increased mammary secretory tissue and decreased mammary adipose tissue (Sejrsen et al., 1983). Dairy cows selected for higher milk production had greater GH response to TRH (Kazmer et al., 1986) and GHRH (Lukes et al., 1989) than unselected control cows. Most recently, we observed that GH response to GHRH in mature beef cows is positively related to milk production (Auchtung et al., 2001a). In the present study, GH response to GHRH in weanling heifers was positively related to their subsequent milk production, as estimated by D_2O dilution and the WSW methods.

The high dose (4.5 μg/100 kg BW) of GHRH in this study was not related to any estimates of milk production. These results are in contrast to our previous study (Auchtung et al., 2001a), in which the higher dose of GHRH was related to estimates of milk production, but the low dose (1.5 μg GHRH/100 kg BW) was not. This is likely due to the differences in the age of the animals at the time of the GHRH challenge. In the previous study, mature cows (2 to 5 yr of age) were challenged, whereas, in the present study, heifers were challenged at weaning (7.1 mo of age). There is strong evidence that there is a decrease in circulating GH and a lower GH responsiveness to GHRH as cattle age (Plouzek and Trenkle, 1991; Lapierre et al., 1992). Therefore, the relative youth of the heifers at the time of challenge in the present study would likely be associated with greater overall sensitivity to GHRH. The higher dose of 4.5 μg GHRH/100 kg BW would yield maximal responses and thus limit the animal-to-animal variability critical to a discrimination of differences among heifers. Regardless, the present data provide further evidence that the response to GHRH can reliably predict milk production in beef cattle.

Deuterium oxide is a stable isotope of water that crosses body barriers at the same rate as water and rapidly becomes uniformly distributed in total body water (Rudolph et al., 1988). Total body water has been estimated using D_2O dilution in piglets (Rudolph et al., 1988), beef steers (Arnold et al., 1985), dairy cows (Andrew et al., 1995), lactating ewes (Cowan et al., 1979), and lactating goats (Dunshea et al., 1990). Dilution of D_2O in body water over time has been useful in estimating the milk intake of a number of species, including pigs (Prawirodigdo et al., 1990a), horses (Oftedal et al., 1983), sheep (Dove and Freer, 1979), reindeer, and caribou (McEwan and Whitehead, 1971). Results of the present study suggest that D_2O dilution accurately estimates milk intake in free-ranging cattle as well. In the present study, a slight difference between milk production as estimated by WSW and the D_2O dilution methods was observed, with the estimations of WSW being lower. This is in accordance with other studies (Prawirodigdo et al., 1990b; Pettigrew et al., 1985) and is likely due to salivary and metabolic losses that were not recorded during WSW.

The D_2O dilution method described in this study appears to be a definite improvement, in terms of accuracy and adaptability, over standard milk yield-estimation methods in the beef cattle industry, such as WSW and machine milking. Although the cost of buying and analyzing D_2O may be considered expensive, it must be compared to the numerous disadvantages of the standard methods. Weigh-suckle-weigh requires repeated, extensive separation of the offspring from their dams and the timing of the measurements is critical (Williams et al., 1979). In addition, WSW is not easily adapted for use on large farms and is labor-intensive and time-consuming. Machine milking requires the animals to become acclimated to the equipment prior to the experiment. If time is not taken to accustom the animals to the equipment, safety of the handlers and the animals is at risk. Further, the stress on the dam could disrupt the milk ejection reflex and the offspring would be stressed for the duration of the time it takes to milk the dam (Beal et al., 1990). Because D_2O dilution requires only infrequent blood sampling and no machine or timed milking, the dam and calf are exposed to less stress.

Implications

Milk production of the beef cow has tremendous impact on the weaning weight of the calf; however, stan-

dard techniques of milk-production estimation have several disadvantages. Growth hormone response to growth hormone-releasing hormone in beef heifers at weaning is able to predict first-lactation milk production. In addition, deuterium oxide dilution estimates milk production in cattle accurately and easily with little disturbance to the cows or calves and could be readily adapted for use in the typical beef production setting.

Literature Cited

Andrew, S. M., R. A. Erdman, and D. R. Waldo. 1995. Prediction of body composition dairy cows at three physiological stages from deuterium oxide and urea dilution. J. Dairy Sci. 78:1083–1095.

Arnold, R. N., E. J. Hentges, and A. Trenkle. 1985. Evaluation of the use of deuterium oxide dilution techniques for determination of body composition of beef steers. J. Anim. Sci. 60:1188–1200.

Auchtung, T. L., D. S. Buchanan, C. A. Lents, S. M. Barao, and G. E. Dahl. 2001a. Growth hormone response to growth hormone-releasing hormone in beef cows divergently selected for milk production. J. Anim. Sci. 79:1295–1300.

Auchtung, T. L., E. E. Connor, S. M. Barao, L. W. Douglass, and G. E. Dahl. 2001b. Use of growth hormone response to growth hormone-releasing hormone to determine growth potential in beef heifers. J. Anim. Sci. 79:1566–1572.

Beal, W. E., D. R. Notter, and R. M. Akers. 1990. Techniques for estimation of milk yield in beef cows and relationships of milk yield to calf weight gain and postpartum reproduction. J. Anim. Sci. 68:937–943.

Connor, E. E., S. M. Barao, E. Russek-Cohen, and G. E. Dahl. 2000. A two-sample method for assessing growth hormone response to growth hormone-releasing hormone challenge: use as a predictor of gain in beef bulls. J. Anim. Sci. 78:1954–1959.

Cowan, R. T., J. J. Robinson, J. F. D. Greenhalgh, and I. McHattie. 1979. Body composition changes in lactating ewes estimated by serial slaughter and deuterium dilution. Anim. Prod. 29:81–87.

Dove, H., and A. Axelsen. 1979. Estimation of milk consumption in beef calves using a tritiated water dilution technique. Aust. J. Exp. Agric. Anim. Husb. 19:666–672.

Dove, H., and M. Freer. 1979. The accuracy of tritiated water turnover rate as an estimate of milk intake in lambs. Aust. J. Agric. Res. 30:725–729.

Dunshea, F. R., A. W. Bell, and T. E. Trigg. 1990. Body composition changes in goat during early lactation estimated using a two-pool model of tritiated water kinetics. Br. J. Nutr. 64:121–128.

Jenkins, T. G., and C. L. Ferrell. 1992. Lactation characteristics of nine breeds of cattle fed various quantities of dietary energy. J. Anim. Sci. 70:1652–1660.

Kazmer, G. W., M. A. Barnes, R. M. Akers, and R. E. Pearson. 1986. Effect of genetic selection for milk yield and increased milking frequency on plasma growth hormone and prolactin concentration in Holstein cows. J. Anim. Sci. 63:1220–1227.

Lapierre, H., C. Farmer, C. Girard, and P. Brazeau. 1992. Effect of age and intake on growth hormone kinetics in dairy heifers. Domest. Anim. Endocrinol. 9:199–207.

Lukes, A. J., M. A. Barnes, and R. E. Pearson. 1898. Response to selection for milk yield and metabolic challenges in primiparous dairy cows. Domest. Anim. Endocrinol. 6:287–298.

Macfarlane, W. V., B. Howard, and B. D. Siebert. 1969. Tritiated water in the measurement of milk intake and tissue growth of ruminants in the field. Nature 221:578–581.

McEwan, E. H., and P. E. Whitehead. 1971. Measurement of the milk intake of reindeer and caribou calves using tritiated water. Can. J. Zool. 49:443–449.

Oftedal, O. T., H. F. Hintz, and H. F. Schryver. 1983. Lactation in the horse: Milk composition and intake by foals. J. Nutr.113:2196–2206.

Pettigrew, J. E., A. F. Sower, S. G. Cornelius, and R. L. Moser. 1985. A comparison of isotope dilution and weigh-suckle-weigh methods for estimating milk intake by pigs. Can. J. Anim. Sci. 65:989–992.

Plouzek, C. A., and A. Trenkle. 1991. Growth hormone parameters at four ages in intact and castrated male and female cattle. Domest. Anim. Endocrinol. 8:63–72.

Prawirodigdo, S., R. H. King, A. C. Dunkin, and H. Dove. 1990a. Evaluation of techniques for estimating milk production by sows. 1. Deuterium oxide dilution method for estimating milk intake by piglets. Aust. J. Anim. Sci. 3:135–143.

Prawirodigdo, S., R. H. King, A. C. Dunkin, and H. Dove. 1990b. Evaluation of techniques for estimating milk production by sows. 2. Estimating the milk consumption of piglets by the deuterium oxide dilution and weigh-suckle-weigh methods. Aust. J. Anim. Sci. 3:143–149.

Rudolph, B. C., T. S. Stahly, and G. L. Cromwell. 1988. Estimation of body composition of neonatal pigs via deuterium oxide dilution: Validation of technique. J. Anim. Sci. 66:53–61.

Sejrsen, K., J. T. Huber, and H. A. Tucker. 1983. Influence of amount fed on hormone concentrations and their relationship to mammary growth in heifers. J. Dairy Sci. 66:845–855.

Williams, J. H., D. C. Anderson, and D. D. Kress. 1979. Milk production in Hereford cattle. I. Effects of separation interval on weigh-suckle-weigh milk production estimates. J. Anim. Sci. 49:1438–1448.

Yates, N. G., W. V. Macfarlane, and R. Ellis. 1971. The estimation of milk intake and growth of beef calves in the field by using tritiated water. Aust. J. Agric. Res. 22:291–306.